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Antimicrobial susceptibility trends among *Escherichia coli* and *Shigella* spp. isolated from rural Egyptian paediatric populations with diarrhoea between 1995 and 2000

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ABSTRACT

Antimicrobial susceptibility testing was performed on 3627 isolates of *Escherichia coli* and 180 isolates of *Shigella* spp. collected in rural locations from 875 Egyptian children with diarrhoea between 1995 and 2000. The cumulative rates of resistance for *E. coli* and *Shigella* spp. were high (respectively, 68.2% and 54.8% for ampicillin, 24.2% and 23.5% for ampicillin–sulbactam, 57.2% and 42.5% for trimethoprim–sulphamethoxazole, and 50.9% and 75.4% for tetracycline). Non-enterotoxigenic *E. coli* (NETEC) isolates had a consistently higher level of antimicrobial resistance than did enterotoxigenic *E. coli* (ETEC) isolates. Trend testing showed significant decreases in resistance to ampicillin, ampicillin–sulbactam and tetracycline among all *E. coli* isolates. Increasing rates of resistance were observed for trimethoprim–sulphamethoxazole in ETEC isolates and *Shigella* spp., but not in NETEC isolates. Low levels of resistance were observed for all other antimicrobial agents tested. Overall, high levels, but decreasing trends, of resistance to commonly used antimicrobial agents were detected among isolates of *E. coli* and *Shigella* spp. from children in rural Egypt.

Keywords Antibiotic resistance, diarrhoea, Egypt, *Escherichia coli*, resistance, *Shigella* spp.

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INTRODUCTION

Infectious diarrhoea continues to cause significant morbidity and mortality among children worldwide [1]. This problem is especially acute in developing countries, where *c.* 25% of all deaths in children aged <5 years are associated with an acute infectious diarrhoeal episode [1]. Two of the most important bacterial agents of childhood diarrhoea in developing countries are enterotoxigenic *Escherichia coli* (ETEC) and *Shigella* spp. [2,3]. Globally, *c.* 400 million ETEC-associated episodes of diarrhoea occur annually, with an estimated 700 000 deaths [4]. Those most at risk of ETEC-associated diarrhoea in developing countries are children aged <5 years, travellers and deployed military personnel [5–7]. In addition to ETEC, an estimated 165 million episodes of infection with *Shigella* spp. occur annually, with *c.* 1.1 million deaths [3].

Numerous reports have described antibiotic resistance among pathogenic and non-pathogenic bacteria [8–11], and have documented increasing numbers of treatment failures associated with pathogens showing decreased susceptibility to commonly prescribed antimicrobial agents [8,12–16]. Although there have been few previous reports focusing on diarrhoeal disease, the high frequency with which antibiotics are used empirically to treat diarrhoeal disease suggests that there might also be high rates of treatment failure associated with enteric infections [17,18].

Worldwide hospital-based surveillance systems (e.g., Alexander Project, SENTRY, MYSTIC [19])

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have demonstrated that antibiotic resistance continues to increase in both developed and developing countries. However, such surveillance systems are based primarily on sterile-site recovery of pathogens from hospitalised patients. In contrast, the primary objective of the present study was to assess the level of antibiotic resistance and temporal trends among *Shigella* spp. and ETEC recovered from children in Egypt with community-acquired diarrhoea. A secondary objective was to compare the antibiotic resistance rates found in ETEC and non-ETEC isolates during the study period.

MATERIALS AND METHODS

Study population and surveillance

Enteropathogens were recovered during 1995 and 2000 from Egyptian infants and children residing in the Abu Homos district, *c.* 40 km south-west of Alexandria, Egypt, during one of three prospective diarrhoeal studies conducted by US Naval Research Unit No. 3 (NAMRU-3). The first (February 1995 to February 1998) was a paediatric longitudinal diarrhoeal surveillance study [20]; the second was an on-going birth-cohort diarrhoeal study started in February 1998; and the third (October 1998 to September 2001) was a phase III ETEC vaccine trial. Informed consent was obtained from the parents of all subjects, and the research was conducted in compliance with all applicable Federal Regulations governing the protection of human subjects in research.

For all three studies, study investigators visited each enrolled child at home twice-weekly. If a 'loose, liquid or bloody stool' was reported by the parents, a faecal sample and rectal swab were collected, and the child was referred to a study physician for evaluation and possible treatment [21]. In addition, a questionnaire was completed for each subject at the time of specimen collection to obtain data regarding demographics, severity of illness, food and drink consumption, illness among family members, previous antibiotic use and other putative exposure variables.

Rectal swabs were inoculated into Cary–Blair transport medium and, together with the faecal samples, were stored in a cool box and transported to a field laboratory. Fresh stools were inoculated into buffered glycerol saline, matched with the rectal swabs, and refrigerated at 4°C before transport (also at 4°C) to the microbiology laboratory at NAMRU-3.

Laboratory isolation and identification

Standard laboratory procedures were used for the isolation of enteric pathogens, with the identity of isolates being confirmed with the API 20E system (Analytab Products, New York, NY, USA). Commercially available antisera (Becton Dickinson, Sparks, MD, USA) were used to serotype all recovered *Shigella* isolates. Up to five individual colonies of presumptive *E. coli* from each agar plate were assayed for the production of heat-labile toxin (LT) and heat-stable toxin (ST) with two different enzyme immunoassays [22,23]. Isolates that produced toxin(s) were defined as ETEC, while non-toxin producers were defined as non-enterotoxigenic *E. coli* (NETEC).

Isolate selection

As a substantial number of *E. coli* isolates were collected over the 6-year study period, a sampling scheme was used to select an unbiased sample for antibiotic susceptibility testing. First, the isolates were stratified according to their originating study site (three-level stratification). Then, within the study site, stratification was by month and year of collection. Finally, isolates from each month were selected by simple random sampling. As there were significantly fewer isolates of *Shigella* spp., all isolates collected between January 1995 and December 2000 were tested.

Antimicrobial susceptibility testing

Antimicrobial susceptibilities were tested by the disk diffusion method [24], and were interpreted according to National Committee for Clinical Laboratory Standards guidelines [25] as either susceptible, intermediate or resistant. For analysis, the intermediate and resistant categories were grouped together as 'non-susceptible'. Multiresistance was defined as non-susceptibility to at least three families of antibiotics, including ampicillin, trimethoprim–sulphamethoxazole and tetracycline. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.

Statistical analysis

Differences in proportions for binary variables were analysed with the Mantel–Haenszal chi-square test. To assess trends in susceptibility across the study period, logistic regression was used to determine whether antibiotic resistance (binary outcome) was dependent on the year of collection, while adjusting for potential confounders. To control for parameter estimate bias associated with repeated measures among the study cohorts, a generalised estimating equation regression model was used, which reported parameter estimates and p values. Statistical significance was set at $p < 0.05$ (two-sided). All data were analysed with SAS v. 8.0 software (SAS Institute, Cary, NC, USA).

RESULTS

Study population

In total, 874 children participated in the three major cohort studies. Of these, 365 children were enrolled during 1995–2000 as part of the diarrhoea surveillance study, with the remaining children participating in either the birth-cohort study ($n = 197$) or the ETEC vaccine efficacy trial ($n = 312$).

Overall, 3178 representative isolates of *E. coli* were tested during the 6-year period of the present study (Table 1). During this period, 180 isolates of *Shigella* spp. were recovered from 193 children (Table 2). There was significant variation

Table 1. General characteristics of children from whom *Escherichia coli* was isolated

Characteristic	1995	1996	1997	1998	1999	2000
Median age (months)[a]	13	13	14	8	14	12
Age in months (%)						
< 6	20.5	17.8	18.5	27.5	7.7	18.5
6–11	23.9	25.2	25.7	49.8	27.3	28.7
12–23	44.3	35.2	33.5	20.8	60.7	37.8
> 23	11.3	21.8	22.3	1.9	4.3	15.0
Male (%)	55.3	58.5	52.5	47.8	48.7	54.6
Previous antibiotic use (%)[b]	5.4	8.5	9.5	13.5	7.6	9.1
Total isolates (*n*)	591	540	579	207	700	561
ETEC (%)	44.2	41.5	48.9	32.4	32.3	25.3

[a]Mean age was calculated based on the age at the time of specimen collection.
[b]Antibiotic taken within the 3 days before collection of the specimen.

Table 2. General characteristics of children from whom *Shigella* spp. were isolated

Characteristic	1995	1996	1997	1998	1999	2000
Median age (months)[a]	16	15	15	7	15	15
Age in months (%)						
< 6	5.2	0	10.0	42.9	4.3	0
6–11	18.2	25.0	15.0	42.9	17.4	20.0
12–23	59.7	41.7	50.0	14.3	78.3	60.0
> 23	16.9	33.3	25.0	0	0	20.0
Male (%)	57.1	41.7	70.0	71.4	34.8	80.0
Previous antibiotic use (%)[b]	0	0	0	0	8.7	0
Total isolates (*n*)	87	12	23	7	46	5
Shigella dysenteriae	12.6	16.7	17.4	14.3	19.6	20.0
Shigella flexneri	55.2	83.3	65.2	58.7	50.0	60.0
Shigella boydii	0	0	0	0	8.7	20.0
Shigella sonnei	32.2	0	17.4	0	21.7	0

[a]Mean age was calculated based on the age at the time of specimen collection.
[b]Antibiotic taken within the 3 days before collection of the specimen.

in the recovery of *Shigella* spp., but the predominant species was *Shigella flexneri*, accounting for ≥50% of the isolates recovered each year. The isolation rate of *Shigella dysenteriae* remained fairly constant throughout the 6-year period.

The median age and the categorised age groups of the children yielding *E. coli* and *Shigella* spp. did not demonstrate significant year-to-year variation, with the exception of 1998 (the year in which the birth-cohort study began enrolling newborn infants). The gender distribution did not change significantly among the group from which *E. coli* was isolated (Table 1), but there was variation among the group yielding *Shigella* spp. (Table 2). Overall, there was an initial increase in use of antecedent antibiotics, peaking (13.5% of children) for the *E. coli* group in 1998, followed by decreases in 1999 (8%) and 2000 (9%) (Table 1). For the group from whom *Shigella* spp. were isolated, antecedent antibiotic use was reported only during 1999 (8.7% of children).

Table 3. Antibiotic resistance found in *Escherichia coli* isolates from 1995 to 2000, stratified according to toxin production

Antibiotic	Toxin production	1995	1996	1997	1998	1999	2000	Trend test (β/p)
Total	ETEC	323	258	330	72	255	126	
	NETEC	369	336	304	157	570	497	
				% resistance				
Ampicillin	ETEC	64.1	69.8	57.6	73.6	58.2	64.1	− 0.04/0.2
	NETEC	72.1	78.0	79.3	80.9	61.9	69.6	− 0.1/< 0.0001
Ampicillin–sulbactam	ETEC	17.3	22.1	17.3	19.4	14.5	9.6	− 0.1/0.01
	NETEC	24.9	34.8	42.1	35.7	23.2	22.9	− 0.09/0.004
Trimethoprim–sulphamethoxazole	ETEC	39.6	60.1	57.9	65.3	47.8	52.1	0.05/0.1
	NETEC	57.2	67.6	68.8	73.3	53.0	57.3	− 0.07/0.004
Tetracycline	ETEC	36.5	61.6	36.1	43.1	35.3	36.5	− 0.08/0.02
	NETEC	55.8	63.7	66.1	61.8	50.4	53.7	− 0.08/0.0009
Amikacin	ETEC	0	0	0	0	0	0	
	NETEC	0	0	1.3	0	0.4	0	
Aztreonam	ETEC	0.3	0.8	1.5	2.8	1.2	1.3	
	NETEC	0.8	0.8	0.6	4.6	0.6	1.2	
Cefepime	ETEC	0	0	0	0	0	0.6	
	NETEC	0	0	0	0	0	0	
Ceftriaxone	ETEC	0	0	0	0	0	0.6	
	NETEC	0.3	0.6	0.7	0	0	0	
Gentamicin	ETEC	0	0	0	0	0	0.6	
	NETEC	0.3	0.6	1.3	0	0	0.2	
Nalidixic acid	ETEC	0.3	1.6	0.6	0	0	0.6	
	NETEC	1.1	1.2	4.0	1.9	1.4	2.8	
Ciprofloxacin	ETEC	0	0	0	0	0	0	
	NETEC	0	0.3	0.7	0.6	0.2	0.6	
Ticarcillin–clavulanic acid	ETEC	5.3	9.7	4.6	8.3	4.7	7.1	
	NETEC	19.2	11.3	19.4	20.4	11.1	9.3	
Ceftazidime	ETEC	0.3	0.8	0.3	1.4	0.4	0	
	NETEC	0.3	0	1.0	1.3	0.2	0	
Imipenem	ETEC	0	0	0	0	0	0	
	NETEC	0	0	0	0	0	0	
Multiresistant[a]	ETEC	25.7	41.9	25.8	30.6	19.6	27.6	− 0.08/0.03
	NETEC	48.8	50.3	53.6	51.6	33.5	33.8	− 0.1/< 0.0001

ETEC, enterotoxigenic *E. coli*; NETEC, non-enterotoxigenic *E. coli*.
[a]Resistant to at least three families of antibiotics, including ampicillin, trimethoprim–sulphamethoxazole and tetracycline.

Table 4. Antibiotic resistance found in isolates of *Shigella* spp. from 1995 to 2000

Antibiotic	Organism	1995	1996	1997	1998	1999	2000	Trend test (β/p)
	Total	87	12	23	7	46	5	
	S. boydii	0	0	0	0	4	1	
	S. dysenteriae	11	2	4	1	9	1	
	S. flexneri	48	10	15	6	23	3	
	S. sonnei	28	0	4	0	10	0	
				% resistance				
Ampicillin	*S. boydii*	–	–	–	–	50.0	0	
	S. dysenteriae	0	0	100	100	11.1	100	
	S. flexneri	97.9	90.0	60.0	33.3	78.3	66.7	– 1.2/0.002
	S. sonnei	3.7	–	0	–	10.0	–	
Ampicillin–sulbactam	*S. boydii*	–	–	–	–	0	0	
	S. dysenteriae	0	0	0	0	0	0	
	S. flexneri	52.1	50.0	26.7	0	26.1	66.7	– 0.2/0.1
	S. sonnei	0	–	0	–	0	–	
Trimethoprim–sulphamethoxazole	*S. boydii*	–	–	–	–	100	0	
	S. dysenteriae	0	0	0	100	33.3	100	
	S. flexneri	14.6	50.0	20.0	16.7	47.8	66.7	0.2/0.6
	S. sonnei	96.3	–	75.0	–	90.0	–	
Tetracycline	*S. boydii*	–	–	–	–	75.0	0	
	S. dysenteriae	0	0	100	0	22.2	100	– 1.0/0.009
	S. flexneri	95.8	100	60.0	33.3	79.9	66.7	
	S. sonnei	96.3	–	75.0	–	100	–	
Amikacin	*S. boydii*	–	–	–	–	0	0	
	S. dysenteriae	0	0	0	0	0	0	
	S. flexneri	4.2	0	6.7	0	0	0	
	S. sonnei	0	–	0	–	0	–	
Aztreonam	*S. boydii*	–	–	–	–	0	0	
	S. dysenteriae	9.1	0	0	100	0	0	
	S. flexneri	4.2	0	0	0	0	0	
	S. sonnei	0	–	0	–	0	–	
Cefepime	*S. boydii*	–	–	–	–	0	0	
	S. dysenteriae	0	0	0	0	0	0	
	S. flexneri	0	0	0	0	0	0	
	S. sonnei	0	–	0	–	0	–	
Ceftriaxone	*S. boydii*	–	–	–	–	0	0	
	S. dysenteriae	0	0	0	100	0	0	
	S. flexneri	0	0	0	0	0	0	
	S. sonnei	0	–	0	–	0	–	
Gentamicin	*S. boydii*	–	–	–	–	0	0	
	S. dysenteriae	0	0	0	0	0	0	
	S. flexneri	2.1	0	0	0	0	0	
	S. sonnei	0	–	0	–	0	–	
Nalidixic acid	*S. boydii*	–	–	–	–	14.3	0	
	S. dysenteriae	0	0	0	0	0	0	
	S. flexneri	0	0	0	0	0	0	
	S. sonnei	0	–	0	–	0	–	
Ciprofloxacin	*S. boydii*	–	–	–	–	0	0	
	S. dysenteriae	0	0	0	0	0	0	
	S. flexneri	0	0	0	0	0	0	
	S. sonnei	0	–	0	–	0	–	
Ticarcillin–clavulanic acid	*S. boydii*	–	–	–	–	0	0	
	S. dysenteriae	0	0	0	0	0	0	
	S. flexneri	0	0	0	0	4.4	33.3	
	S. sonnei	0	–	0	–	0	–	
Ceftazidime	*S. boydii*	–	–	–	–	0	0	
	S. dysenteriae	0	0	0	0	0	0	
	S. flexneri	0	0	0	0	0	0	
	S. sonnei	0	–	0	–	0	–	
Imipenem	*S. boydii*	–	–	–	–	0	0	
	S. dysenteriae	0	0	0	0	0	0	
	S. flexneri	0	0	0	0	0	0	
	S. sonnei	0	–	0	–	0	–	
Multiresistant[a]	*S. boydii*	–	–	–	–	0	0	
	S. dysenteriae	0	0	0	0	25.0	0	
	S. flexneri	14.6	40.0	20.0	16.7	34.8	66.7	0.3/0.4
	S. sonnei	3.6	–	0	–	0	–	

[a]Resistant to at least three families of antibiotics, including ampicillin, trimethoprim–sulphamethoxazole and tetracycline.

Antibiotic susceptibility trends

Overall, there were modest annual changes in the percentage of isolates resistant to antibiotics that are commonly available and used in developing countries against both *E. coli* and *Shigella* spp. (Tables 3 and 4). There was minimal resistance to quinolones (nalidixic acid, ciprofloxacin), advanced-generation cephalosporins (ceftazidime, ceftriaxone, cefepime), carbapenems (imipenem), monobactams (aztreonam) and aminoglycosides (amikacin, gentamicin). The lack

of annual resistance data for these antibiotics precluded valid trend analysis.

Decreasing trends of antibiotic resistance were noted among ETEC isolates for ampicillin, ampicillin–sulbactam, tetracycline and the multiresistance phenotype (Table 3). The only agent that demonstrated an increasing resistance trend among ETEC isolates was trimethoprim–sulphamethoxazole, but this trend was not significant. Among NETEC isolates, there was a consistent decrease in the resistance trends across the study period for ampicillin, ampicillin–sulbactam, trimethoprim–sulphamethoxazole, tetracycline and the multiresistance phenotype (Table 3). Among the isolates of *Shigella* spp., only *S. flexneri* had enough data points to enable valid trend analysis; this showed increasing resistance to trimethoprim–sulphamethoxazole, and the multiresistance phenotype, but decreasing resistance to ampicillin, ampicillin–sulbactam, and tetracycline (Table 4).

DISCUSSION

In agreement with reports from other developing countries [9,26–32], the present study demonstrated very high rates of resistance in *E. coli* and *Shigella* spp. to antibiotics that are commonly available in Egypt. These resistance rates remained fairly stable throughout the 6-year study period. Previous studies have demonstrated a strong correlation between antibiotic resistance and high rates of antibiotic use, with a reduction in antibiotic use being followed by a reduction in resistance [33–35]. In contrast, the present study found low antecedent antibiotic use, perhaps indicating that the results reflect community-based exposure to and acquisition of resistant enteric flora, as opposed to the development of antibiotic resistance following individual consumption of antibiotics. Walson *et al.* [36] demonstrated in Nepal that community-based factors (e.g., population density, community and/or regional consumption of antibiotics, distance from allopathic health care, and other unmeasured factors) provided strong, or stronger, predictors of antibiotic resistance carriage than the consumption of antibiotics by individuals. The data in the present study also supported a 'community exposure' model, and the population studied had open access to antibiotics from local pharmacies. Therefore, it is quite possible that significant antibiotic misuse was occurring in the population, thereby selecting and maintaining large numbers of resistant bacteria in the community. The observed lack of resistance to antibiotics used rarely in the community supports this model.

The data revealed several statistically significant decreasing trends in antibiotic resistance in the period 1995–2000, but these should be viewed in the context of a rural developing region in which high levels of antibiotic resistance among enteric pathogens were common in the community. There was no indication of a dramatic increase or decrease in rates of resistance among isolates of *E. coli* and *Shigella* spp. during the 6-year period. However, there appeared to be a slight increase in low-level resistance to newer antibiotics during the last 2 years of the study (Table 3).

The regression trend analysis was adjusted, according to age and previous antibiotic use because of potential confounding of these two variables. Age was associated with antibiotic resistance, in that younger children were more likely than older children to yield resistant strains. It was also demonstrated that age was associated with previous antibiotic use, with younger children receiving antibiotic treatment more often for a variety of infectious diseases. Similarly, Howard *et al.* [33] reported that the rate of resistance in isolates of *E. coli* from urinary tract infections decreased as the age of children increased. Previous antibiotic use among the present sample was difficult to assess, but the limited data indicated that there was an association between previous antibiotic use and either the recovery of resistant isolates or age.

The higher levels of resistance in NETEC, compared to ETEC, was somewhat disconcerting, as NETEC strains, forming part of the normal intestinal flora, could serve as quasi-permanent reservoirs of transmissible antibiotic resistance determinants [9,37]. The continual shedding of antibiotic-resistant *E. coli* from the normal flora into an environment where selection exists for antibiotic resistance could result in community-wide cycling of resistant non-pathogenic enteric flora among the population. A previous study [38] concluded that previous antibiotic use by patients in long-term care institutions explained the recovery of antibiotic-resistant *E. coli*, but that conditions which facilitate spread may be more important in sustaining high resistance in such

an environment. The overall resistance found in *Shigella* spp. reflected the rates of resistance found in other developing countries [26,29–32,39,40], but could not be analysed further because of the relatively small number of isolates recovered in the present study.

A significant limitation of the present study was that it was not possible to determine antibiotic resistance rates among isolates from adolescents and adults. The observed rates may be over-estimates of the true community-based antibiotic resistance rates for this region, as antibiotic use tends to decrease with age. Other limitations were the unavailability of information regarding other variables (e.g., previous hospitalisation, other underlying disease) that are associated with the isolation of antibiotic-resistant bacteria, and the use of isolates from children participating in three separate studies. However, the children studied were all residents of the same village, and standardised methods (i.e., active surveillance, sample and data collection, laboratory testing, etc.) were used for all three studies.

In developing countries, antibiotic resistance has been linked with inappropriate use of antibiotics, over-the-counter availability of antibiotics, lack of health care personnel with adequate training, poor-quality drugs and poor sanitary conditions [41]. Continued surveillance of antibiotic resistance, especially in community-based populations within developing countries, is crucial to alleviate morbidity and mortality among those living in and visiting these countries.

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